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13. ABSTRACT (Maximum 200 words)

The feasibility of noninvasive, transcutaneous, near infrared spectral measurement of tissue pH in humans was experimentally demonstrated in this Phase I SBIR program. A prototype near infrared spectrometer instrument was developed that allowed for measurement of deep tissue pH in the kidney areas of 28 normal male and female volunteers. Changes in pH were artificially induced by hyperventilation and breath holding. Near infrared estimates of tissue pH values were compared with reference laboratory values of venous blood pH for 6 blood samples of each patient. Near infrared estimates of pH demonstrated an accuracy of ± 0.03 pH in comparison with reference laboratory values. A Phase II SBIR program is recommended to develop a portable, near infrared field instrument to measure human tissue pH in a battlefield combat casualty care or a civilian emergency medical environment.

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1.0 Introduction

In both military combat casualty care and in various civilian emergency medical scenarios, there is a need for noninvasive biochemical sensors for diagnosing and monitoring the status of trauma/shock victims. An important example of such a critical biochemical variable is tissue pH. A real-time noninvasive measurement of tissue pH would be of extreme value to attending medical personnel in battlefield or emergency medical environments.

Current diagnostic medical technology for human pH measurement is primarily limited to laboratory analysis of extracted blood samples. In battlefield or emergency medical environments, such an approach is limited by:

1. The time lag between blood extraction and laboratory analysis. Delayed measurements of tissue pH are of limited value in a fast-changing trauma/shock situation.

2. Laboratory analysis may be extremely difficult or even impossible under the stress and disorder of battlefield conditions.

3. Blood pH values are not necessarily representative of tissue pH values, particularly under extreme acidic or alkaline conditions.

For all of the above reasons, an on-site, real-time, noninvasive measurement of tissue pH under battlefield or emergency medical conditions would represent a dramatic improvement of current diagnostic/monitoring medical technology.

Previous developments of noninvasive measurements of body tissue-based biochemical parameters have been extremely limited. The most important thrust has been the search for a noninvasive measurement instrument of blood glucose for diabetics. Noninvasive glucose sensor development has inspired a wide variety of approaches to the problem [Peura 84]. The two primary approaches have been electrochemical and optical. One electrochemical approach was enzymatic in nature using the enzyme glucose oxidase that converts glucose in the presence of oxygen to hydrogen peroxide and gluconic acid

$$Glucose + O_2 \rightarrow Glucose\ Oxidase \rightarrow Gluconic\ Acid + H_2O_2$$

Immobilized glucose oxidase was coupled with an oxygen-sensitive polarographic electrode that measured the change in oxygen concentration caused by the glucose conversion. A second electrode was added later to account for variations in the amount of oxygen present [Clark 62]. Other versions of the electroenzymatic approach have been attempted more recently without, however, any notable end-product resulting [Wingard 82][Soeldner 76].

A second electrochemical design is based on the direct electrocatalytic conversion of glucose on a platinum electrode [Lewandowski 82]. Other investigators have developed implantable versions of this platinum electrode-based glucose sensor [Lerner 82].

A major difficulty with all of the electrode-based glucose sensors is the need for very frequent recalibration that is not practical in a glucose-monitoring home-testing or an emergency medical environment. This need results from the instability of the immobilized enzyme and the fouling of the sensor surface under physiological conditions.

A number of opto-chemical approaches to glucose measurement have also been developed that depend on some enzymatic or catalytic reaction that changes light intensity of a given color or a set of specified wavelengths outside the visible range [Schultz 82][Pilosof 82]. These sensors suffered from the same instabilities as the electrochemical glucose sensors.

The instability of sensors based on immobilized chemical reactions has diverted attention to optical design approaches that operate independently of any induced chemical reaction. One such approach is based on the measurement of glucose in the aqueous humor of the eye [March 82] that has been shown to be proportional to the concentration of glucose in blood. By measuring the optical rotation of a polarized beam of light directed laterally through the anterior chamber of the eye, it is possible to obtain a quantitative measurement of glucose [Peura 84]. These optical rotations are very small, however, and are often obscured by the presence of large corneal birefringence. Also, for home testing, such a form of optical measurement may be considered more invasive than current blood sample testing technology.

A more direct optical measurement approach is based on near infrared spectrometry. Near infrared spectrometry has been widely used for many years to determine the chemical composition of grains and other agricultural products [Osborne 86]. It is based on the varying spectral "fingerprints" of different chemical compounds in the near infrared spectral region [Weyer 85]. A significant difficulty encountered in the application of this technology is the exceedingly large number of chemical compounds that exhibit absorption spectra in the near infrared region. This problem has been partially overcome through the use of advanced statistical, signal processing and pattern recognition techniques that allow for accurate estimation of analyte concentrations in complex chemical matrices.

Near infrared spectrometry also has recently been applied to the noninvasive measurement of glucose, cholesterol and other blood analytes [Dahne 85][Lodder 89][Schlager 89][Rosenthal 91]. Most descriptions of the technology as applied to glucose are in the patent literature as referenced above. To the knowledge of the author of this proposal (Schlager), only Schlager and Rosenthal have developed prototype near infrared instruments and tested them on a significant number of patients.

This somewhat extensive discussion of the application of near infrared (NIR) spectrometry to glucose and other blood analytes has been necessary to establish the foundation for the present developments of NIR for tissue pH. Biotronics and the principal investigator (Schlager) have been major participants in this work, and this near infrared experience with glucose, urea and especially the blood electrolytes (sodium, potassium, calcium and others) provided the "springboard" for the Phase I tissue pH program reported here.

Tissue pH measurement is essentially an electrolyte measurement in near infrared terms

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since it affects the NIR spectrum in the same manner as sodium, calcium or potassium. Unlike organic compounds, such as glucose and urea, which have intrinsic near infrared absorption patterns, pH (hydrogen ion concentration) has secondary NIR spectral effects through its distortion of the NIR water spectrum. Water is the dominant NIR absorber, and any modification of its spectrum has a major impact on the overall NIR absorption spectrum. This distortion of the dominant water spectrum results from the formation of so-called coordination compound between the ion of interest (H^+) and water molecule [Buijs 63][Choppin 63]. This coordination compound by its distortion of the water spectrum generates a new spectrum that is dependent in its shape and amplitude on the value of pH in the solution. Other electrolytes such as sodium (Na^+) and calcium (Ca^{++}) have similar effects, forming different coordination compounds, each with its own NIR spectrum. The literature relating to NIR spectra of coordination compounds is extremely sparse with little that is comprehensive in nature published beyond the Buijs and Choppin papers referenced above.

Empirically, however, Biotronics has had extensive experience demonstrating the efficacy of noninvasive near infrared analysis of electrolytes. A recent SBIR program with the Naval Medical Research and Development Command, in which 5 electrolytes were successfully determined, is documented in a report by the principal investigator [Schlager 93]. The foundation established by the Navy program led to the tissue pH program for the Army reported here.

The overall objective of the Phase I research/development program was to demonstrate the feasibility of near infrared spectral measurement of deep tissue pH in humans as an aid in trauma/shock diagnosis and treatment. The specific objectives of the Phase II program were defined in the Phase I proposal as follows:

1. Demonstrate the mathematical relationship between NIR spectra and pH levels in complex synthetic, biological matrices and human tissue;

2. Demonstrate the capability of NIR spectral measurements to estimate pH at varying tissue depths from surface to deep tissue;

3. Demonstrate the sensitivity and accuracy of NIR bench prototype instrumentation;

4. Demonstrate the efficacy of selected pattern recognition algorithms to convert NIR spectra into accurate pH measurements; and

5. Demonstrate the design potential for a lightweight, portable NIR/pH instrument suitable for military field use.

Basic feasibility was demonstrated based on preclinical test results of near infrared spectral measurements of 28 normal subjects with varying tissue pH values conducted at the Department of Emergency Medicine of the University of Kentucky College of Medicine. Tissue pH changes measured in the lower back (kidney) area were induced by hyperventilation (alkalosis) and breath holding (acidosis) while simultaneous blood sampling and NIR spectral measurements were performed. The pH value of all patients was measured with an accuracy of ±0.05, and all patients were classified correctly as

either:

1. Normal (7.35 - 7.45)
2. Low pH (less than 7.35)
3. High pH (greater than 7.45)

with a classification accuracy of 100%. The lowest pH recorded was 7.29 and the highest 7.50. This performance bodes well for the future success of the program since more extreme pH changes are to be expected in an actual emergency or combat casualty care situation.

Some of the individual specific objectives were modified under Army direction during the course of the Phase I program. Objective 2 was modified in that emphasis was placed on deep tissue measurements under instructions from Dr. Fred Pearce, the Contracting Officer's Representative. Otherwise, the specific objectives were accomplished with the exception of the human tissue experiments which were curtailed at the end of the program for lack of time. All resources were concentrated on the preclinical instrumentation and human testing program. The sensitivity and accuracy of the prototype instrumentation was successfully demonstrated, and powerful pattern recognition algorithms were developed for pH measurement.

The general approach used to accomplish these objectives involved a combination of laboratory experimentation and preclinical testing of healthy humans under artificially-induced stress. The primary approach emphasized human testing based on the use of a prototype near infrared spectrometer developed during the Phase I program. This instrument was designed, built and then employed for the testing of the 28 individuals referenced in the paragraph above. A secondary approach employed laboratory experimentation using simulated biological matrices to verify the relationship between pH and near infrared absorption spectra and to provide for a wider range of pH variation than was possible with the healthy subjects in the human trials.

2.0 Project Narrative

Research and Development in Phase I were carried out in six primary areas.

1. *In vitro* laboratory experimentation of VNIR/pH spectral measurements with simulated biological matrices.

2. Development of a prototype VNIR/pH data collection instrument.

3. Formulation of preclinical human test protocol in conjunction with the University of Kentucky Medical Center.

4. Development of pattern recognition algorithms to convert VNIR spectra into accurate pH measurements.

5. Preclinical testing of 28 volunteer subjects at the University of Kentucky Medical Center.

6. Analysis of preclinical test data to determine the accuracy of VNIR pH measurement.

In vitro Laboratory Experimentation

In vitro laboratory experimentation was very helpful early in the Phase I program in verifying the changes in VNIR spectra that occur with changing pH. Strong absorbance changes were observed at 973 nm with pH variations in the range of 6.85 to 7.26. Less strong but equally significant absorbance changes were recorded in the 850 nm waveband. Both sets of results are recorded in the tables below.

973 nm	
pH	Absorbance
6.85	2.0164
6.93	2.0540
7.03	2.0655
7.14	2.0768
7.25	2.0800
7.39	2.0840
7.54	2.0862
7.76	2.0958

850 nm	
pH	Absorbance
6.85	0.2457
6.93	0.2477
7.03	0.2497
7.14	0.2512
7.25	0.2522
7.39	0.2550
7.54	0.2560
7.76	0.2603

The absorbance changes of 0.079 at 973 nm and 0.015 at 850 nm (from low to high pH) are quite significant and indicators of a measurable phenomenon in this spectral region. These changes were confirmed later by laboratory experimentation and with preclinical human testing. All of these laboratory measurements were performed on a Perkin Elmer Lambda 9 UV-VIS-NIR spectrophotometer. Additional runs were carried out at wavebands of 973 nm and 921 nm with substantially similar results.

Later laboratory tests were conducted in which 41 biological matrix solutions with varying levels of pH as well randomly varying levels of bovine serum and sodium chloride were used. The objective of this testing was to determine the ability of the new pattern recognition algorithms to accurately determine pH levels in the presence of interference from varying protein levels (in the serum) and changing concentrations of sodium chloride. As reported in the July 15th monthly technical progress report, early testing on the Perkin Elmer Lambda 9 spectrophotometer did not provide sufficient precision to allow for accurate NIR/pH analysis. During August and September, extensive efforts were made to achieve optimal scan precision in these experiments. Scan speed was reduced, and sensitivity (signal gain) and response time (low filter bandwidth) were optimized. In addition, twenty five (25) scans were performed on each of the 41 samples. Unfortunately, the time-consuming nature of this testing process resulted in changes in the serum protein that nullified the attempts at precision measurement. The changes in the absorption pattern of the protein over the life of the experiment far exceeded the degree of precision required for accurate pH measurement. It is now quite clear that a fast-scanning photodiode array spectrometer will be required to execute in vito experiment with the precision needed for accurate *in vitro* experimentation with simulated biological matrices including animal or human tissue. The prototype spectrometer described later in this proposal for preclinical testing in Phase II should also be quite suitable for *in vitro* experimentation with simulated biological matrices.

Despite the limitations encountered with traditional laboratory instrumentation, the *in vitro* experiments in Phase I did verify the basic relationship between pH level and near infrared light absorption spectra. These experiments also identified the NIR spectral pattern of pH for guidance in the preclinical data analysis. Finally, the requirements for more complex *in vitro* simulation of human tissue pH were strongly clarified by the Phase I experience. While it is realized that human trials are the final basis for evaluating the efficacy of noninvasive NIR/pH measurement technology, a parallel laboratory experimental program is invaluable in providing guidance in the final development of the instrument.

Prototype Instrument Development

Modification of the Phase I objectives under Army technical direction, emphasizing deep Tissue pH measurement exclusively, led to a change in direction for the prototype instrument design. Since shallow depth pH measurement was no longer an immediate requirement, the original instrument design could be replaced by a design that optimized for the 800-1100 nm range. This very near infrared (VNIR) spectral region provides for the maximal depth of tissue penetration. Accordingly, the new design shown in Figure 1 was developed for the preclinical human pH testing.
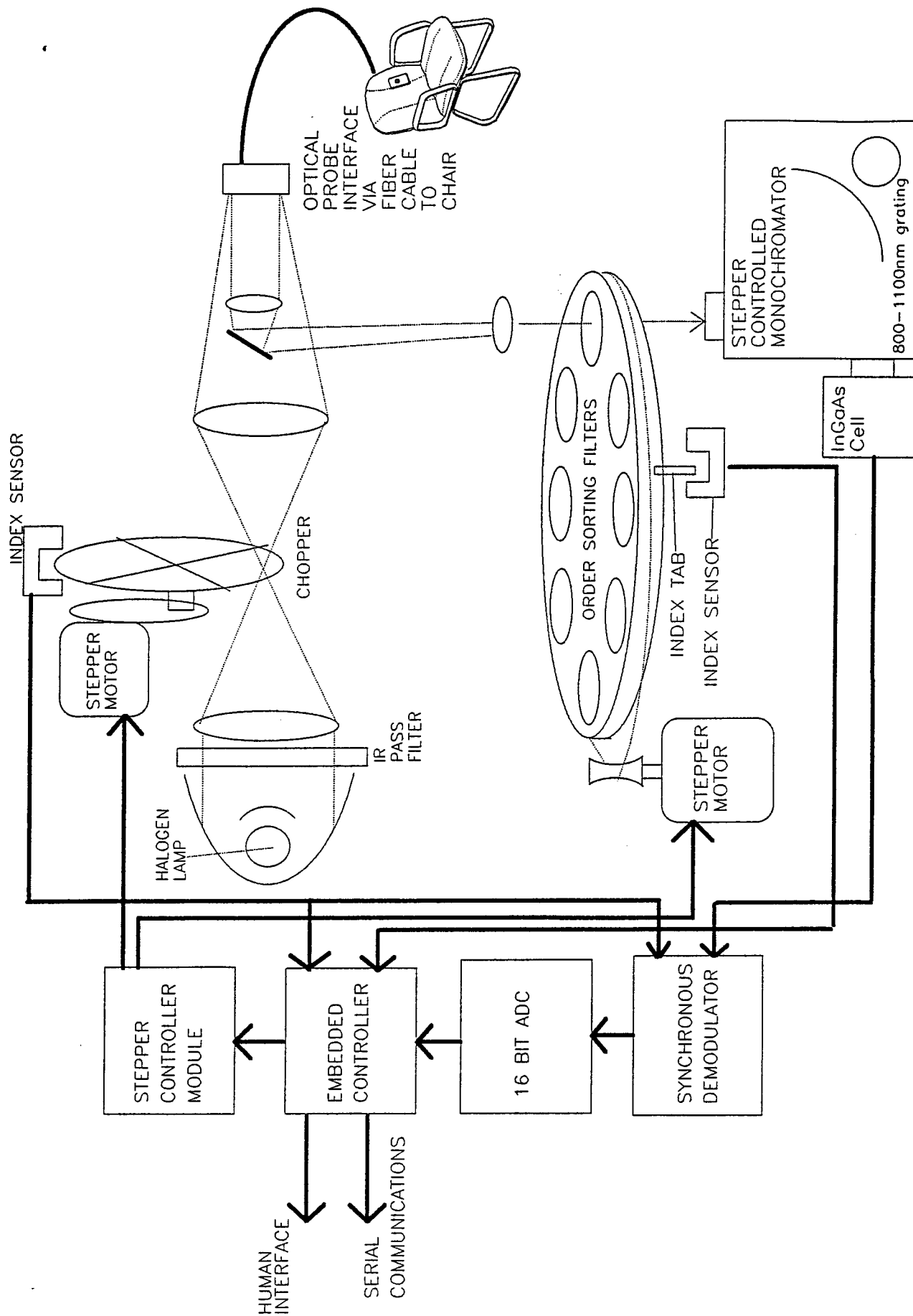
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FIGURE 1: NEAR INFRARED DATA COLLECTION TISSUE pH SPECTROMETER – FUNCTIONAL DIAGRAM

The new VNIR/pH design incorporated the following features:

1. A monochromator optimized for the 800-1100 nm spectral region.

2. A Photodetector cell that represented a significant signal-to-noise improvement over the originally-proposed lead selenide cell but which could still scan throughout the 800-1700 nm region. This indium gallium arsenide cell performed well throughout the human test sequence.

3. A fiber probe optimized for lower back (kidney area) pH measurement and embedded in a comfortable chair for test subject convenience.

The instrument shown in Figure 1 utilizes a halogen light source and a stepper motor-driven chopper assembly to generate modulated light which is transmitted through a fiber bundle to the skin surface where the probe in contact with the skin transmits light into the epidermis, dermis and beyond. Some of this light is back scattered back into the probe and received for transmission back through an order sorting filter (to minimize harmonic light effects) and to a monochromator which scans the 800-1100 nm range with output to an InGaAs photodetector the signal of which is detected in a synchronous demodulator (lock-in amplifier) after which it is converted to digital form for processing in the computer. An embedded controller transmits the data serially to an attached personal computer.

The instrument was designed, constructed and checked out in the May-August time period. The instrument was tested on personnel at Biotronics using the pH test protocol before it was shipped to the University of Kentucky for preclinical testing on August 11, 1995.

Test Protocol Development

The test protocol for the NIR measurement of tissue pH went through a number of modifications, alterations and extensions before it was approved by both the University of Kentucky Medical IRB and the Army Human Use and Regulatory Affairs Division. The challenge was to develop a protocol that was both technically feasible and medically acceptable.

The basic concept underlying the protocol was to induce pH changes in healthy adult subjects by hyperventilation and breath holding while continually sampling venous blood samples using a heparin lock.

Three sets of scans were performed on each patient while sitting in the test chair and interfaced in his/her lower back kidney area to the fiber probe. Each scan set consisted of 2 30 second scans under the three following conditions:

1. Normal breathing
2. Hyperventilation (alkalosis)
3. Breath holding (acidosis)

The instrument scan began in each instance 30 seconds after the subject assumed the condition, so that each test period was 60 seconds in length. A 1 cc venous blood sample was taken during each of the 6 scans. These blood samples were to be analyzed for pH in the laboratory using the blood gas machine pH method.

After a final series of revisions, the experimental test protocol and the consent form were approved by UK Medical (August 9) and the Army on August 10. The complete protocol and consent form are included in Appendix I.

Preclinical Testing

The prototype NIR instrument was shipped on Friday, August 10 and arrived in good order on Monday, August 14. After some minor maintenance, the instrument was checked out and approved for preclinical testing.

Testing began at 0630 hours on Tuesday, August 15th. The first patient was tested at 0726 hours and the last patient at 2058 hours. Twenty eight (28) patients were tested using the test protocol previously described, and 168 spectra were collected. All but four (4) of the spectra were deemed useable. All data were collected for transfer on 7 diskettes as well as the data on the Winchester (hard) disk drive. One set of data was kept at UK Medical and the other two (diskette and system) were sent to Biotronics in Wisconsin.

Pattern Recognition Algorithms

The development of methods suitable for the detection of abnormal pH levels must be robust with respect to variations between patients and must be able to deliver suitable sensitivity and specificity on the basis of relatively few exemplary (learning set) measurements. This problem can be solved through the design of a pattern recognition system focused on distinguishing and classifying the spectral VNIR measurements related to pH concentration. The pattern recognition system necessitates three main developmental steps consisting of feature extraction, realization of a classification model and the selection of prototype measurements.

Feature extraction is used to eliminate the redundancy of information contained in spectral measurements, reduce the magnitude of noise and interferences and enhance the characteristics of the measurements related to the target analyte. The diverse methods of well established feature extraction techniques include principal component analysis (PCA). Although the mathematical calculations are involved, the central idea behind PCA is to reduce the dimensionality of a large number of interrelated variables (wavelengths) while retaining the information that distinguishes one sample from another. This is achieved by transforming to a new set of variables, the principal components (PCs), which are uncorrelated, and which are ordered so that the first few retain most of the variation present in all of the original variables. A small subset of the principal components are selected as representative of the data's significant characteristics or features either through statistical measures, correlation analysis or crossvalidation.

The optimal classification model defined by Bayesian Theory requires perfect knowledge of the *a priori* probabilities of the population. Conversely, given a relatively small sample

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set of a complex target population and no *a priori* information, the k-nearest neighbor (KNN) algorithm has been shown to produce exceptional classification models. The KNN algorithm provides a computationally simple nonparametric procedure for the assignment of a class to an input pattern based on a small set of prototype observations (the learning set). In its simplest form, a sample is assigned to the class of its nearest neighbor based on the Euclidean distance. This is extended to the k-nearest neighbors with the sample being assigned to the class that is represented by a majority among the k closest samples. The value selected for k is dependent upon the application and the number of learning set observations or prototypes. As the size of the learning set approaches infinity, increasing k will cause the error rate to approach the optimal Baysian rate.

When a disparity exists between the number of samples in each of the learning set classes or when overlap exists among the classes, the performance of the KNN classifier is significantly degraded. The use of fuzzy set theory to overcome these and other problems has produced a fuzzy k-nearest neighbor (FKNN) algorithm that is a small but significant modification to the KNN approach. Fuzzy classification does not consider class membership in the conventional "crisp" manner but rather specifies a degree of class membership. This is of particular advantage in applications in which class membership is not clearly established or where, as in the pH study, it is of benefit to assign a certainty of class membership.

The basis of the FKNN algorithm is to assign membership as a function of the input pattern's distance from its k-nearest neighbors. Each of the k-nearest neighbors assigns the input pattern an estimate of the degree of membership in its respective class. These are combined to produce an overall estimate of class membership and the probability of class membership. The degree of membership in the class assigned by a particular prototype pattern to an input is based upon a fuzzy membership function that is characterized by rapid nonlinear decrease in influence as distance increases. The influence of a prototype therefore decreases with its distance from the input pattern.

A crucial aspect of the FKNN algorithm is the selection of a set of prototypes representative of the perspective population the classifier will be applied to. The problem is one of selecting from a set of clinical measurement containing outliers and noisy measurements those samples most representative of all future measurements. Genetic algorithms are an effective solution to complex optimization problems involving a large set of permutations such as this. A fundamental advantage GAs furnish over traditional approaches is the ability to optimize complex problems that cannot be expressed mathematically by a continuous criterion function.

As the name suggests, GAs optimize by applying the general principles of natural selection and the mechanics of genetics. The algorithm operates on a diverse population or set of possible solutions to an optimization problem. At each training iteration, termed a "generation", the possible solutions are evaluated according to their performance and assigned a "fitness level" indicating their likelihood of survival to the next generation. Through random selection, members that are more fit tend to "reproduce" and form a new population of offspring by combining with other highly fit solutions.

The learning set optimization problem can be approached by defining each member of the

GA population as a set of samples used to create the FKNN model. Evaluation of the population members is then determined by the ability of the each to classify the entire sample set. The result of the genetic algorithm optimization process is a set of prototype patterns most suitable for classifying the entire sample set.

This advanced three component pattern recognition system comprised of PCA feature extraction, FKNN classification and learning set optimization through genetic algorithms was successfully developed and implemented during Phase I. The results are summarized in the following section.

Analysis of Test Results

The results of the preclinical testing at UK Medical exceeded the expectations of the principal investigator and his associates at Biotronics on the Phase I pH/VNIR project. The results are particularly noteworthy because of the relatively small changes in pH that were induced by hyperventilation and breath holding. With a pH of 7.29 on the low end and 7.50 on the high end, the maximum change from the normal 7.35-7.45 range was 0.06. Despite this extremely small pH variation, VNIR measurements were able to detect each of the abnormal measurements on the low and high end with perfect classification accuracy. The 100% classification accuracy reported here in a small sample of patients undoubtedly involves a element of chance, but it does indicate the potential of very near infrared (VNIR) spectrometry in noninvasive pH measurement. There seems little question that tissue pH influences the VNIR spectrum, and that these variations are being measured.

Aside from the quality of the VNIR spectral measurements which should be significantly improved in the new Phase II instrument designs, the favorable Phase I results were undoubtedly strongly influenced by the fuzzy K nearest-neighbor (FKNN) classifier algorithm used in data analysis. Continued intensive development of these pattern recognition algorithms is planned for Phase II.

Data

Clinical data from 28 subjects (158 samples) were obtained using the VNIR spectrometer instrument. Associated with each subject were 6 reflective light intensity measurements in the range 850-1000 nm, 6 laboratory based blood pH measurements and individual values for age, weight and height.

Preprocessing

The raw reflective intensity data were normalized by dividing by the low reflective standard and then by the average intensity of wavelengths 800-850 nm. The 977 wavelength measurements were reduced to 196 by low-pass filtering followed by frequency decimation.

A principal components analysis was performed on the entire data set of 144 samples and the ratio of weight/height was calculated as an approximation of body fat.

The sample set was divided into low (16 samples), normal (136 samples) and high (6 samples) pH regions divided at the 7.35 and 7.45 pH levels.

Pattern Recognition

A correlation analysis on the principal components was performed to determine the 5 principal components most correlated with blood pH. A fuzzy KNN classification model was designed using 5 neighbors and an exponential weighting factor related to the Euclidean distance.

The key to the FKNN model applied to signals embedded in noise is the determination of the best set of exemplar samples characterizing the distribution of the population. This problem was solved by using a genetic algorithm to select the near-optimal set of samples for estimating the class of the entire sample set.

The genetic algorithm operated on 200 members, employed 3 types of crossover and has a mutation rate 0f 0.05%. Evaluation was performed by using the selected set of samples to estimate the class of the entire sample set in a leave-one-out approach. Fitness was defined as the average of sensitivity and specificity. Sensitivity and specificity are defined below:

TP - True Positive
   Correct indication of abnormal pH

TN - True Negative
   Correct indication of normal pH

FP - False Positive
   Incorrect indication of abnormal pH

FN - False Negative
   Incorrect indication of normal pH

Sensitivity Sen = TP/(TP + FN)
   Given a population with a low or high pH, what percent of the abnormal population will be detected?

Specificity Spec = TN/(TN + FP)
   Given a population with a normal pH, what percent of the population will be indicated as normal?

Note: Low pH and high pH were considered as separate classes, so that pattern recognition was formulated as a 3-class problem - low, high and normal.

Results

The results of Fuzzy KNN classification are shown in Tables 1, 2, 3 and 4 below. Tables 1 and 2 relate to low/normal pH classification, and Tables 3 and 4 tabulate high/normal pH

classification. In Table I, the experimental results rely entirely on the VNIR spectra as the input variables (features). These variables are expressed as the 5 most-correlated principal components, which are essentially a weighted VNIR spectra with the weights proportional to the correlation with pH estimation. The sensitivity of 75% reflects that 12 of the 16 abnormal (low) pH individuals were classified correctly. There were also only 20 false positives out of 128 normal samples, so that specificity was recorded at a respectable 84%. The overall accuracy of classification was 83%.

In Table II, the weight/height ratio of each individual was added as a variable to VNIR spectral vector. A significant explanation for individual VNIR absorption differences lies in fat content. The back kidney area used in these tests is a notable location for fat content differences between individuals. The addition of the weight/height factor significantly improves the sensitivity of the system, so that all 16 low abnormals are classified correctly (100% sensitivity). Specificity is also improved with only 16 false positives for a specificity of 87%. Overall accuracy rose to 89%.

In Tables III and IV, the same tabular descriptions apply for the high/normal classification with Table III based on spectra alone, and Table IV incorporating the weight over height feature. Again, the combination of VNIR spectral and weight/height feature variables resulted in a sensitivity of 100%. Overall accuracy was 95%.

Table I	
Input Variables	5 Principal Components
Number of Samples	144
Number of Selected Samples	40 (10 low)
Correct Classifications	120
Incorrect Classifications	24
Overall Accuracy (Percent Correct)	83%
True Positive (low)	12
True Negatives (normal)	108
False Positive	20
False Negatives	4
Sensitivity	75%
Specificity	84%
Low/Normal pH Classification	
VNIR Spectra	

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Table II	
Input Variables	5 Principal Components, Normalized weight over height
Number of Samples	144
Number of Selected Samples	58 (12 low)
Correct Classifications	128
Incorrect Classifications	16
Overall Accuracy (Percent Correct)	89%
True Positive (low)	16
True Negatives (normal)	112
False Positive	16
False Negatives	0
Sensitivity	100%
Specificity	87%
Low/Normal pH Classification	
VNIR Spectra + Weight/Height Feature	

Table III	
Input Variables	5 Principal Components
Number of Samples	142
Number of Selected Samples	
Correct Classifications	132
Incorrect Classifications	10
Overall Accuracy (Percent Correct)	93%
True Positive (high)	4
True Negatives (normal)	128
False Positive	8
False Negatives	2
Sensitivity	67%
Specificity	94%
High/Normal pH Classification	
VNIR Spectra	

Table IV	
Input Variables	5 Principal Components, normalized weight over height
Number of Samples	142
Number of Selected Samples	
Correct Classifications	135
Incorrect Classifications	7
Overall Accuracy (Percent Correct)	95%
True Positive (high)	6
True Negatives (normal)	129
False Positive	7
False Negatives	0
Sensitivity	100%
Specificity	95%
High/Normal pH Classification	
VNIR Spectra + Weight/Height Feature	

Note: The FKNN algorithm was later extended to direct pH value estimation. Test results are recorded in Appendices I and II.

3.0 Conclusions

The overall objective of demonstrating the feasibility of noninvasive, near infrared deep tissue measurement of pH in humans was definitely accompished in Phase I. Results of the human testing of 28 individuals under induced stress for pH change, supported the capability of near infrared spectra to measure tissue pH. Measurement capability at the ±0.05 pH level was demonstrated by the successful classification of each individual as low, normal or high pH with a maximum change of 0.06 on the low side and 0.05 on the high side. Later extension of the FKNN algorithm to pH estimation resulted in pH estimation accuracy performance of ±0.026. These results are documented in Appendices I and II. Feasibility of VNIR/pH was further verified by the ability of the very near infrared (VNIR) spectrometer instrument to detect the pH changes for each individual as he/she moved from a normal to high or a low pH condition.

The results of the Phase I experience at Biotronics strongly support the feasibility of developing a compact, portable, lightweight VNIR spectrometer instrument that could noninvasively measure deep tissue pH in a military combat casualty care or civilian emergency medicine environment. The program to accomplish this goal is detailed in the previously submitted Phase II proposal. Only the highlights of that Phase II program will be summarized here.

To realize the potential for VNIR/pH measurement for combat casualty care, the following research/development program tasks must be accomplished in Phase II.

1. A new VNIR photodiode array-based spectrometer instrument must be developed for both laboratory and human testing.

2. This new instrument must be tested with both tissue samples in the laboratory and patients in a clinical environment with extreme as well as normal values of tissue pH.

3. The differences between tissue pH and blood pH must be determined by a separate swine study which will allow for simultaneous invasive and noninvasive measurements of tissue pH.

4. All of the above experience must then be factored into the development of a VNIR/pH spectrometer field instrument that is compact, portable, lightweight, reliable and easy to use and is very suitable for the military combat casualty care environment.

5. This field instrument in 4 above must then be demonstrated in an actual field medical environment.

4.0 References

1. [Abitol 82] Abitbol, M.M., "Fetal Heart Rate and Tissue pH changes Associated with Repetitive Aortic Occlusion in the Pregnant Dog",Am J. Obset. Gynecol, 143: 430-439, 1982.

2. [ADA 93] Lipsett, L., "Statistics: Prevalence, Incidence, Risk Factors and Complications of Diabetes," American Diabetes Association Bulletin, April 9, 1993.

3. [Bhandare 93] Bhandare, P. "Glucose Determination in Simulated Blood Serum Solutions by Fourier Transform Infrared Spectroscopy: Investigaation of Spectral Interferences", Vibrational Spectroscopy, 6: 363-378, 1994.

4. [Bhat 80] Bhat, R., "Continuous Tissue pH Monitoring in Critically Ill Neonates", Fetal and Neonatal Medicine, 97: 445-450, 1980.

5. [Boyer 94] J. Boyer et al., Proceedings of the SPIE, 2389, Pg. 103 (1994), and M.R. Ostermeyer and S.L. Jacques, Proceedings of the SPIE, 2389, Pg. 98 (1994).

6. [Buijs 63] Buijs, K., "Near-Infrared Studies of the Structures of Water. I. Pure Water", The Journal of Chemical Physics, Vol. 39, No. 8, 1963.

7. [Cha 86] Cha, S. and Gabele, P., "Study on Infrared Gas-Filter Correlation Spectrometer for Measuring Low-Concentration Methanol Gases," Optical Engineering, vol. 25, no. 12, December, 1986.

8. [Choppin 63] Choppin, G.R., "Near-Infrared Studies of the Structure of Water. II. Ionic Solutions", The Journal of Chemical Physics, Vol. 39, No. 8, 1963.

9. [Clark 62] Clark, L. and Lyons, C., "Electrode System for Continuous Monitoring in Cardiovascular Surgery," Ann. N.Y. Acad. Sci., vol. 102, pp. 29-45, 1962.

10. [Couch 71] Couch, N.P., "Muscle Surface pH as an Index of Peripheral Perfusion in Man", Annals of Surgery, 173: 173-183, 1971.

11. [Dahne 85] Dahne, C. and Cross, D.,"Spectrophotometric Method and Apparatus for the Noninvasive Determination of Glucose in Body Tissues", European Patent Application, Publ. Number 0160768, 1985.

12. [Das 84] Das, J.B., "Percutaneous Monitoring of Tissue pH with a Polymer Membrane pH Sensor", in: Fetal and Neonatal Physiological Measurements, Proc. 2nd International Conference, Oxford, United Kingdon, 1984.

13. [Dia 93a] "The Race is on for a Noninvasive Sensor," Diabetes Interview, April, 1993.

14. [Dia 93b] Diabetes Interview, November, 1993.

15. [Dodson 85] Dodson, M.G., "Continuous Intrapatrum Fetal Tissue pH Monitoring", The

American College of Obstetricians and Gynecologists, pp. 43-44, 1985.

16. [Dunn 78] Dunn, L.K., "The Relationship Between Tissue and Arterial pH in Hypercarbic Rabbits", Arch Gynecol, 226: 31-38, 1978.

17. [Dunteman 89] Dunteman, G. H., "Principal Components Analysis", Sage Publications, 1989.

18. [Filler 71] Filler, R.M. "Muscle Surface pH: A New Parameter in the Monitoring of the Critically Ill Child", Pediatrics, 47: 880-885, 1971.

19. [Fusi 81] Fusi, L., "An Evaluation of the Tissue pH Electrode for Fetal Monitoring Using the Fetal Sheep as an Experimental Model", Am J. Obstet. and Gynecol., 140: 953-960, 1981.

20. [Gazzaniga 70] Gazzaniga, A.B., "The Use of Skeletal Muscle Surface Hydrogen Ion Concentration to Monitor Peripheral Perfusion: Experimental and Clinical Results", Surgical Forum, 21: 147-149, 1970.

21. [Gazzaniga 72] Gazzaniga, A.B., "Effects of Dexamethosone and Chlopromazine on Skeletal Muscle Surface Hydrogen Ion Concentration During Cardiopulmonary Bypass: Experimental and Clinical Results", Annals of Surgery, 176: 757-760, 1972.

22. [Kellner 80] Kellner, K.R., "An Evaluation of a Continuous Tissue pH Monitory in the Human Fetus During Labor", Obstet. Gynecol., 55:523, 1980.

23. [Lerner 82] Lerner, H., et al., "Measurement of Glucose Concentration in the Presence of Coreactants with a Platinum Electrode," Diabetes Care, vol. 5, no. 3, pp. 229-237, 1982.

24. [Lewandowski 82] Lewandowski, J., et al., "Amperometric Glucose Sensor: Short-Term In Vivo Test," Diabetes Care, vol 5, no. 3, pp. 238-294, 1982.

25. [Lodder 89] Lodder, R. A., et al., "Assessment of the Feasibility of Determination of Cholesterol and Other Blood Constituents by Near Infrared Reflectance Analysis," Talanta, vol. 36, no. 112, pp. 193-198, 1989.

26. [March 82] March, N. F., "Noninvasive Glucose Monitoring of the Aqueous Humor of the Eye: Part I Measurement of Very Small Optical Rotation," Diabetes Care, vol. 5, pp. 254-258, 1982.

27. [McCarren 93] McCarren, M., "Intensive Therapy: It Works," Diabetes Forecast, American Diabetes Association, 1993.

28. [Osborne 86] Osborne, B. G., et al., Near Infrared Spectroscopy in Food Analysis, Longman-Wiley, 1986.

29. [Peura 84] Peura, R. A. and Mendelson, Y., "Blood Glucose Sensors: An Overview,"

NSF Symposium on Biosensors, Los Angeles, CA, September 15-17, 1984.

30. [Pilosof 82] Pilosof, D., et al., "Microporous Membrane Flow Cell with Non-Immobilized Enzyme for Chemillumination Determination of Glucose," Anal. Chem., vol. 54, pp. 1698-1701, 1982.

31. [Rithalia 79] Rithalia, S.V.S., "Continuous Moniiitoring of Tissue pH", Br. Med. J., 1:1460-1461, 1979.

32. [Rosenthal 91] Rosenthal, R. D., Paynter, L. N. and Mackle, L. H., "Non-Invasive Measurement of Blood Glucose", U.S. Patent Number 5,028,787, July 2, 1991.

33. [Schlager 89] Schlager, K. J., "Noninvasive Near Infrared Measurement of Blood Analyte Concentrations", U.S. Patent Number 4,882,492, November 21, 1989.

34. [Schlager 93] Schlager, K.J., Transcutaneous Analyte Measure Methods, (TAMM Phase II), Quarterly Progress Report Number 7 to the Naval Medical Research and Development Command, 1993.

35. [Schultz 82] Schultz, J. S., et al., "Affinity Sensor: A New Technique for Developing Implantive Sensors for Glucose and Other Metabolites," Diabetes Care, vol. 5, pp. 245-253, 1982.

36. [Soeldner 76] Soeldner, J. S., et al., NIH Publ., no. 76-854, pp. 267-277, 1976.

37. [Sturbois 77] Sturbois, G., "Continuous Subcutaneous pH Measurement in Human Fetuses. Correlations with Scalp and Umbilical Blood pH", Am. J. Obstet. Gynecol., 128: 901-903, 1977.

38. [Weyer 85] Weyer, L. G., "Near Infrared Spectroscopy of Organic Substances," Applied Spectroscopy Reviews, 21(1), 1-43, 1985.

39. [Wingard 82] Wingard, L. B., et al., "Potentiometric Measurement of Glucose Concentration with an Immobilized Glucose Oxidase/Catalase Electrode," Diabetes Care, vol 5, no. 3, pp. 199-202, 1982.

40. [Young 79] Young, B.K., "The Relationship of Heart Rate Patterns and Tissue pH in the Human Fetus", Am. J. Obstet. Gynecol., 134:685-690, 1979.

Appendix I

An Estimation Extension of the FKNN Algorithm

In the last few days of the Phase I SBIR program after the submission of the Phase II proposal and after the completion of most of the Phase I final report, the Fuzzy K Nearest Neighbor (FKNN) algorithm was extended in capability to include an estimation function. The original FKNN was only capable of classifying the pH of each individual as high-normal-low. Since the changes below the normal range were extremely small, a measurement capability of ±0.05 pH accuracy was inferred, but it was never estimated as such. This new extension of FKNN did, however, permit an estimation of the value of pH based on very near infrared (VNIR) spectra.

The results of this FKNN extension are summarized below:

Measurement Statistics

Number of Samples	= 158
Maximum pH	= 7.5
Minimum pH	= 7.29
Average pH	= 7.39
Range of pH	= 0.21

Estimation Results

Average Absolute Error	= ±.026
Average Percentage Error of Mean	= 0.359735%
Average Percentage Error of Range	= 12.6643%
Statistical t-Value	= 7.81001

An accuracy of 0.026 pH is closely approaching the laboratory reference value accuracy (±0.01 pH), so that these results further support the conclusions of the Phase I SBIR Final Report that very near infrared (VNIR) spectrometry is a feasible approach to noninvasive human tissue pH measurement. The various laboratory reference values of pH together with the FKNN pH estimates for each patient (and each sample) are shown in Appendix II. The rationale for the estimation extension of the FKNN algorithm is provided in the section below.

FKNN Algorithm Estimation Procedure

The utilization of fuzzy set theory in K nearest neighbor classification and estimation is a promising approach to analytically compensate for the complex biological and chemical interactions, the profound differences between subjects and the limited sample population inherent in the current pH detection experiment. This approach, previously summarized in the results section, has been advanced for the purpose of directly estimating pH levels.

The principal technique involved several steps. First, the clinical samples were clustered according to their associated blood PH level and "fuzzified" by assigning each sample a group membership value inversely related to the distance of the samples blood PH level from each of the cluster means. The FKNN algorithm, as described on Page 14, was applied using

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$$P_i(x) = \cfrac{\sum_{j=1}^{K} P_{ij}\left(\dfrac{1}{d_j^2}\right)}{\sum_{j=1}^{K} \left(\dfrac{1}{d_j^2}\right)}$$

where i is the group index, j refers to one of K nearest neighbors, x is the input spectra, d_j is the Euclidean distance of x from the jth nearest neighbor and p_{ij} is the membership of the jth nearest neighbor in the ith class, and P_i is the membership of the input sample into the ith group. The vector P is a set of extracted features of the input sample with each element related to the degree of membership of the sample in each of the classes. The set of features generated by the learning set was related to the blood pH level by multiple linear regression.

The samples used as potential nearest neighbors significantly affect the classification results. To optimize this learning set, a genetic algorithm was applied that selected the optimal set of potential nearest neighbors based on the ability of the set to estimate the blood pH level of all the samples.

Testing of the algorithm was performed through crossvalidation in which each sample was left of and a new fuzzy KNN estimator was designed using all of the remaining samples. Therefore, the learning set includes samples taken from the same patient as the sample that is left out.

The input pattern, x, consisted of 10 principal components of a given sample and was determined by performing principal component analysis on the entire clinical sample set of spectra pre-processed using a low pass filter followed by the second derivative. The 10 Pcs were selected by determining which had the highest correlation to blood pH.

Appendix II

FKNN pH Estimation Summary
Versus Laboratory Reference Values

Sample Number	Patient Number	Lab pH	FKNN pH	Residual
101	18	7.290	7.373	0.083
130	23	7.290	7.360	0.070
131	23	7.310	7.367	0.057
132	23	7.310	7.398	0.088
65	12	7.320	7.361	0.041
66	12	7.320	7.380	0.060
106	19	7.320	7.351	0.031
107	19	7.320	7.350	0.030
118	21	7.320	7.378	0.058
13	3	7.330	7.397	0.067
14	3	7.330	7.393	0.063
100	18	7.330	7.342	0.012
119	21	7.330	7.408	0.078
71	13	7.340	7.339	0.001
72	13	7.340	7.367	0.027
108	19	7.340	7.365	0.025
1	1	7.350	7.343	0.007
15	3	7.350	7.369	0.019
59	11	7.350	7.396	0.046
73	13	7.350	7.354	0.004
83	15	7.350	7.399	0.049
94	17	7.350	7.410	0.060
95	17	7.350	7.397	0.047

102	18	7.350	7.356	0.006
120	21	7.350	7.364	0.014
2	1	7.360	7.385	0.025
25	5	7.360	7.371	0.011
32	6	7.360	7.365	0.005
37	7	7.360	7.370	0.010
43	8	7.360	7.346	0.014
50	9	7.360	7.392	0.032
60	11	7.360	7.418	0.058
84	15	7.360	7.350	0.010
85	15	7.360	7.416	0.056
89	16	7.360	7.393	0.033
90	16	7.360	7.393	0.033
104	18	7.360	7.374	0.014
112	20	7.360	7.350	0.010
137	24	7.360	7.383	0.023
138	24	7.360	7.406	0.046
147	26	7.360	7.402	0.042
19	4	7.370	7.373	0.003
26	5	7.370	7.408	0.038
31	6	7.370	7.433	0.063
33	6	7.370	7.404	0.034
38	7	7.370	7.403	0.033
42	8	7.370	7.387	0.017
67	12	7.370	7.362	0.008
91	16	7.370	7.393	0.023
103	18	7.370	7.369	0.001
105	18	7.370	7.365	0.005

109	19	7.370	7.355	0.015
113	20	7.370	7.358	0.012
114	20	7.370	7.356	0.014
116	20	7.370	7.385	0.015
133	23	7.370	7.408	0.038
136	24	7.370	7.387	0.017
148	26	7.370	7.404	0.034
16	3	7.380	7.380	0.000
20	4	7.380	7.409	0.029
44	8	7.380	7.412	0.032
48	9	7.380	7.384	0.004
49	9	7.380	7.319	0.061
55	10	7.380	7.388	0.008
86	15	7.380	7.412	0.032
96	17	7.380	7.381	0.001
115	20	7.380	7.392	0.012
129	22	7.380	7.417	0.037
153	27	7.380	7.394	0.014
3	1	7.390	7.366	0.024
7	2	7.390	7.384	0.006
22	4	7.390	7.407	0.017
51	9	7.390	7.393	0.003
54	10	7.390	7.429	0.039
97	17	7.390	7.412	0.022
127	22	7.390	7.396	0.006
139	24	7.390	7.399	0.009
154	27	7.390	7.400	0.010
8	2	7.400	7.400	0.000

12	2	7.400	7.411	0.011
17	3	7.400	7.383	0.017
18	3	7.400	7.411	0.011
27	5	7.400	7.404	0.004
53	9	7.400	7.396	0.004
77	14	7.400	7.418	0.018
117	20	7.400	7.354	0.046
126	22	7.400	7.418	0.018
128	22	7.400	7.398	0.002
135	23	7.400	7.387	0.013
149	26	7.400	7.415	0.015
155	27	7.400	7.400	0.000
4	1	7.410	7.389	0.021
21	4	7.410	7.398	0.012
52	9	7.410	7.391	0.019
69	12	7.410	7.384	0.026
70	12	7.410	7.396	0.014
74	13	7.410	7.356	0.054
88	15	7.410	7.414	0.004
93	16	7.410	7.408	0.002
110	19	7.410	7.392	0.018
111	19	7.410	7.385	0.025
121	21	7.410	7.413	0.003
141	24	7.410	7.391	0.019
142	25	7.410	7.415	0.005
10	2	7.420	7.409	0.011
11	2	7.420	7.422	0.002
23	4	7.420	7.395	0.025

24	4	7.420	7.409	0.011
34	6	7.420	7.407	0.013
39	7	7.420	7.344	0.076
61	11	7.420	7.416	0.004
68	12	7.420	7.390	0.030
87	15	7.420	7.414	0.006
98	17	7.420	7.389	0.031
124	22	7.420	7.417	0.003
143	25	7.420	7.403	0.017
145	25	7.420	7.417	0.003
146	25	7.420	7.416	0.004
151	26	7.420	7.413	0.007
152	26	7.420	7.408	0.012
158	27	7.420	7.413	0.007
5	1	7.430	7.392	0.038
6	1	7.430	7.392	0.038
9	2	7.430	7.409	0.021
45	8	7.430	7.403	0.027
76	13	7.430	7.384	0.046
78	14	7.430	7.415	0.015
79	14	7.430	7.425	0.005
92	16	7.430	7.392	0.038
99	17	7.430	7.397	0.033
125	22	7.430	7.407	0.023
134	23	7.430	7.391	0.039
140	24	7.430	7.406	0.024
144	25	7.430	7.413	0.017
150	26	7.430	7.415	0.015

156	27	7.430	7.397	0.033
28	5	7.440	7.402	0.038
35	6	7.440	7.422	0.018
46	8	7.440	7.386	0.054
47	8	7.440	7.414	0.026
75	13	7.440	7.370	0.070
82	14	7.440	7.402	0.038
123	21	7.440	7.405	0.035
157	27	7.440	7.413	0.027
36	6	7.450	7.414	0.036
40	7	7.450	7.429	0.021
41	7	7.450	7.417	0.033
56	10	7.450	7.419	0.031
57	10	7.450	7.408	0.042
58	10	7.450	7.421	0.029
80	14	7.450	7.406	0.044
122	21	7.450	7.404	0.046
29	5	7.460	7.406	0.054
30	5	7.460	7.417	0.043
81	14	7.460	7.404	0.056
62	11	7.470	7.419	0.051
64	11	7.470	7.403	0.067
63	11	7.500	7.409	0.091

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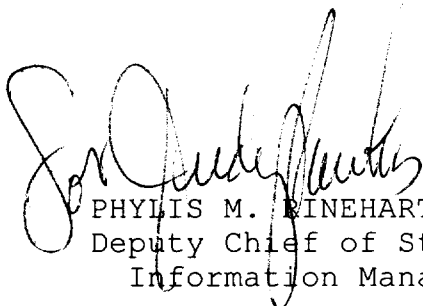SUBJECT: Request Change in Distribution Statement

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